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IN RE APPLICATION OF: Lakshman R. SEHGAL, et al. ART UNIT: 1635

SERIAL NO.: 10/725,013 EXAMINER: Brian A. Whiteman

FILING DATE: December 2, 2003

FOR: EX VIVO AND IN VIVO EXPRESSION OF THE THROMBOMODULIN GENE FOR THE TREATMENT OF CARDIOBASCULAR AND PERIPHERAL VASCULAR DISEASES

DECLARATION UNDER RULE 1.132

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SIR:

I, LAKSHMAN R. SEHGAL, do hereby declare as follows:

I am the first named inventor of the above-identified patent application. I have a Ph.D. degree in Biology, principally covering the fields of Microbiology and Biochemistry, from the Illinois Institute of Technology and have worked closely with cardiovascular, vascular and trauma surgeons for over three decades. I am the primary inventor on six patents related to a hemoglobin based oxygen carrier; a co-inventor on two patents related to emulsion based oxygen carrier; the first named inventor on a pending application related to a platelet preservative solution and first named inventor on two pending applications in the field of gene therapy. I have over 150 publications in peer reviewed journals and chapters in medical textbooks. The

hemoglobin based blood substitute that I developed is the only one in Phase III clinical trials and is being used by ambulances at the site of accidents. I have always focused on inventions that are truly clinically useful.

This invention is related to a method for treating a vascular disease in a mammal, having the steps of: infecting a segment of a blood vessel *ex vivo* using a gutless adenoviral vector which contains a polynucleotide encoding a thrombomodulin protein or its variant and a regulatory element operably linked to said polynucleotide sequence; grafting the virus-treated blood vessel in said mammal, and the thrombomodulin protein or its variant is expressed in the virus-treated blood vessel in an amount sufficient to reduce re-occlusion and /or intimal hyperplasia in the grafted blood vessel.

I have reviewed the Office Action, dated February 21, 2006, and the references cited by the Examiner, namely U.S. Patent No. 6,290,949 to French et al. (hereinafter "French"), U.S. Patent No. 5,981,225 to Kochanek et al. (hereinafter "Kochanek") ,Salyapongse et al. (hereinafter "Salyapongse") He et al. (hereinafter "He"), Sehgal et al. (hereinafter "Sehgal") and Kibbe et al. (hereinafter "Kibbe").

In view of these references, French generally describes an *ex vivo* method of gene therapy for treating a vascular disease using a first generation of adenoviral vector. French does not mention a gutless adenoviral vector. It is clear that the gutless adenovirus vectors of the present Claim 1 are significantly different from the first generation, E1-deleted adenovirus as described in French. Briefly, the first generation virus vectors contain only a deletion in the E1 gene and have following deficiencies: (1) the virus vectors are highly immunogenic and toxic, and (2) have a limited cloning capacity (typically less than five kb) because of the package limit of the adenovirus.

Moreover, neither Salyapongse, He , Schgal, nor Kibbe mentions the gutless adenovirus vectors.

Claim 1 is directed to a medical application of using a gutless adenoviral vector that contains a polynucleotide encoding a thrombomodulin protein or variant for treating a vascular disease. Comparing to the first generation adenovirus vectors, the gutless virus vectors are much less immunogenic and have very little toxicity. The gutless virus vectors have a cloning capacity up to 36 kb. Accordingly, the gutless virus vectors represent a significant improvement over the first generation adenovirus vectors.

It is also known in the field of gene therapy, that the gutless virus vectors are difficult to construct and produce in large quantities. Because the gutless virus carries no viral genes, all the viral structure proteins need to be produced from a helper vector or helper virus. The DNA genome of each gutless virus needs to be optimized in size so that the DNA can be properly packaged into a viral capsid. The purification process of the gutless virus vectors is significantly different than the purification process of the first generation virus. Moreover, the particle/pfu ratio, the infectivity, and the level of transgene expression are all different between the first generation and gutless virus vectors. In addition, although not well characterized in the literature, it is known in the art that certain proteins can interfere with adenovirus production. For example, the apoptotic gene Bax results in premature cell death of the 293 packaging cell line and resulting in minimal yields of recombinant virus (Kagawa et al., Gene Therapy, 7:75-79 (2000)]. Accordingly, to achieve the present invention, a new vector with the specific gene of interest would need to be constructed and produced using a different method. The infection and expressing conditions would need to be reestablished through an enormous experimentation.

Kochanek generally describes a gutless adenoviral vector. However, Kochanek neither mentions treating a disease in a mammal using a gutless adenoviral vector nor does he describe the use of the thrombomodulin gene.

I would like to call the Examiner's attention to the fact that Claim 1 is directed at a medical application of using a gutless adenoviral vector that contains a polynucleotide encoding a thrombomodulin protein or variant for treating a vascular disease. Claim 1 recites the steps of "**infecting a segment of a blood vessel, *ex vivo,*** using a **gutless adenoviral vector** which comprises a polynucleotide encoding a **thrombomodulin protein** or its variant and a regulatory element operably linked to said polynucleotide sequence; ..." To be clinically relevant , the patency of the endothelial cells that line the vessel wall and the primary target of the gutless virus is critical. The disclosed preservative medium is critical to the success of the medical application. Claim 1 further provides the steps of "**grafting** the virus-treated blood vessel in said mammal, wherein said thrombomodulin protein or its variant is expressed in said virus-treated blood vessel in a amount sufficient to **reduce re-occlusion and / or intimal hyperplasia in the grafted blood vessel.**"

Clearly, the present invention is not simply a combination of French with Kochanek, Salyapongse, He , Sehgal and Kibbe, and it offers significant advantages over these references. Specifically, combining the gutless adenoviral vector with the thrombomodulin transgene, a fully patent vein segment with the novel preservative medium, and the *ex vivo* treatment of the vein. All these render the claimed invention particularly well suited for the clinic application for treating occlusive vascular diseases.

I declare that all statements made herein based on my own knowledge are true, and that all statements made herein based on information and belief are believed to be true. I further declare that these statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United State Code, and that willful false statements may jeopardize the validity of the above-referenced patent application and any patent that issues there from.

Date: _____

L. R. Sehgal

Lakshman R. Sehgal, Ph.D

Gene Therapy (2000) 7, 75–79

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VIRAL TRANSFER TECHNOLOGY BRIEF COMMUNICATION

A binary adenoviral vector system for expressing high levels of the proapoptotic gene *bax*

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The bax gene plays a critical role in signaling apoptosis and expression through gene transfer may be valuable in the treatment of a variety of apoptosis-related diseases such as cancer. However, constructing an adenoviral vector expressing a bax gene driven by a constitutive promoter has been difficult, presumably because of the gene's high proapoptotic activity. Here we report a system that induces the expression of the bax gene safely by adenovirus-mediated gene cotransfer. Briefly, the system involves an adenoviral vector containing a human bax cDNA driven by a synthetic promoter consisting of five GAL4-binding sites and a TATA box (GT). This vector expresses a minimal background level of bax protein in cultured mammalian cells thus preventing apoptosis of packaging cells, however, expression of the bax gene can be induced substantially in vitro and in vivo by transferring it into target cells along with an adenoviral vector expressing the transactivator, fusion protein GAL4/VP16. Extensive apoptosis was observed after induction of the bax gene both in cultured human lung carcinoma cells and in the livers of Balb/c mice. Our results suggest that this GAL4 gene regulatory system provides an alternative approach to constructing viral vectors that express potentially toxic genes. Gene Therapy (2000) **7**, 75–79.

Keywords: *gene therapy; apoptosis; bax gene; cell death*

Adenoviral vectors are widely used for *in vivo* gene delivery and gene therapy because of their high transduction efficiencies in a variety of tissues.[1,2] Because of its high transduction efficiencies, adenoviral vector is useful in characterizing the functions and testing the therapeutic effects of a gene *in vivo*. Under most circumstances, constructing an E1-deleted adenoviral vector is quite straightforward. However, constructing an adenoviral vector that expresses a transgene that is potentially toxic to packaging cells can be extremely difficult. For example, constructing adenoviral vectors expressing the fas ligand (*Fas-L*) has been reported to be difficult because of the toxic effect on 293 cells.[3] Although adenoviral vectors expressing *Fas-L* have been constructed and proven to be effective in the treatment of established experimental tumor models, 293 cells that are resistant to Fas-L-induced apoptosis or the caspase inhibitor were used in that particular study to produce such a vector.[4] Alternatively, a stuffer DNA fragment flanked by lox P sites was placed between promoter and *Fas-L* cDNA to prevent transgene expression in 293 cells. Fas-L was then induced by coinfecting target cells with an adenoviral vector expressing were to remove the stuffer fragment.[5] More recently, 293 cells expressing poxvirus *crm A* were reported to produce and expand adenoviral vectors expressing *Fas-L* or *Fas*.[6] However, titers of the vector were low, probably because of incomplete suppression of apoptosis in producer cells, and the method can only be applied to genes whose function is inhibited by crm A.

In the past 2 years, we have experienced difficult in constructing an adenoviral vector expressing the *bax* gene, a member of the Bcl-2 family and an apoptosis promoter,[7,8] driven by the cytomegalovirus promoter. This problem was not solved by using 293 cells stably transfected with the antiapoptotic *bcl-2* gene. Although there are no reports in the literature to document this difficulty, the lack of any literature on the subject in itself suggests that constructing an adenoviral vector expressing the *bax* gene is not an easy task. Moreover, the transfection of cancer cell lines with naked DNA containing the *bax* or *p53* expression cassette has been shown to kill 70–90% of the transfected cells in the case of *bax versus* only 40% in the case of *p53*, suggesting that bax may be more toxic than p53.[9]

We have previously shown that a synthetic GAL4-responsive promoter consisting of five GAL4-binding sites and a TATA box (GT) has low transcriptional activity *in vitro* and *in vivo* when placed in an adenoviral backbone.[10] Moreover, the transgene activity can be substantially induced *in vitro* and *in vivo* by administering this construct along with an adenoviral vector (Ad/PGK-GV16) expressing a GT transactivator, namely, the GAL4-VP16 fusion protein. The low basal level and the inducibility of this binary vector system led us to hypothesize that it might be used to construct adenoviral vectors expressing the proapoptotic gene *bax*. In fact, construction of an adenoviral vector containing GT-Bax (Ad/GT-Bax) is straightforward and induction of the *bax* gene

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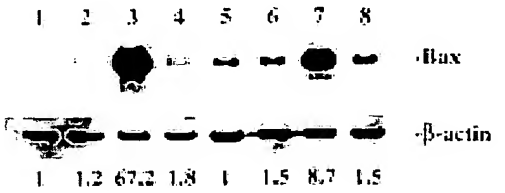
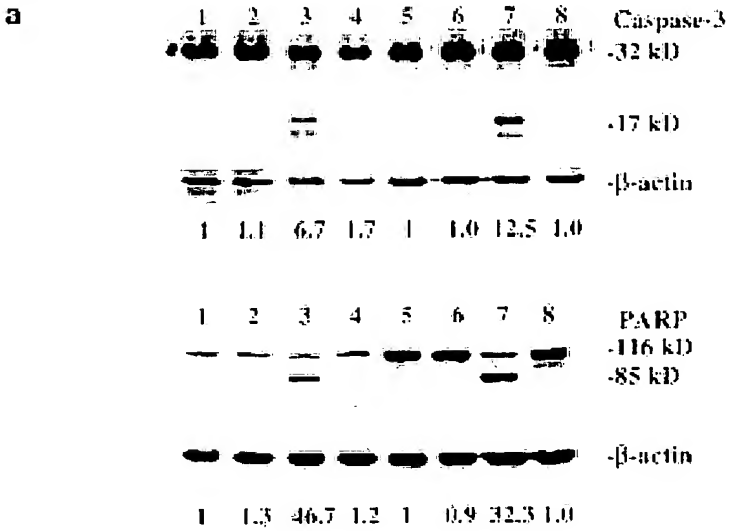


Figure 1 Induction of bax gene expression in H1299 and A549 cells. H1299 (lanes 1–4) and A549 (lanes 5–8) cells were treated with PBS (lanes 1 and 5), Ad/GT-Bax + Ad/CMV-GFP (lanes 2 and 6), Ad/GT-Bax + Ad/PGK-GV16 (lanes 3 and 7), and Ad/GT-LacZ + Ad/CMV-GV16 (lanes 4 and 8). Western blot analysis was performed 24 h after the infection. The bax: β-actin ratio is listed under each lane. These ratios were determined using Optimas software (Media Cybernetics, CA, USA). and are expressed in terms relative to the ratios in PBS-treated cells, which was arbitrarily set at 1.

expression by adenovirus-mediated gene cotransfer is quite effective.

We initially attempted to construct vectors in which the *bax* gene was driven by a CMV promoter, but failed in this after rigorous trials. The problem was not solved by using 293 cells stably transformed and overexpressing the *bcl-2* gene (data not shown). We therefore constructed shuttle plasmids in which *bax* cDNA was driven by GT. Recombinant viral vectors were obtained after a single transfection of 293 cells with pAd/GT-Bax plus a 35-kb *Cla*I fragment from Ad/p53.[11] Virus from a single plaque was expanded in 293 cells and purified twice by ultracentrifugation on a cesium chloride gradient. The vector titer determined by optical obsorbency at A_{260} was 3.3×10^2 viral particles/ml, equivalent to the other E1-deleted vectors, such as Ad/CMV-GFP and Ad/CMV-LacZ. The total yield for Ad/GT-Bax was also the same as for the other E1-deleted vectors produced in our laboratory, about 1.5×10^4 particles per cell. All the vectors used in



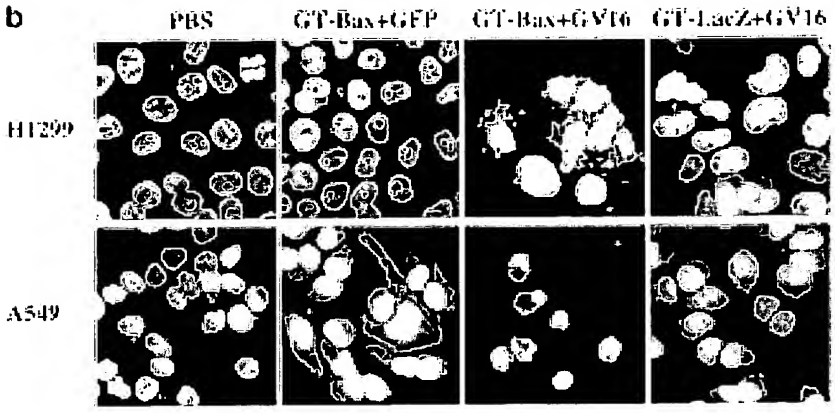


Figure 2 Apoptosis profiles after induction of bax gene expression. (a) Cleavage of caspase-3 and PARP detected in cell samples as described in the legend to Figure 1. The ratio of cleaved to uncleaved enzyme is listed under each lane. These ratios were determined using Optimas software and are expressed in terms relative to the ratio in PBS-treated cells, which was arbitrarily set at 1. (b) Nuclear fragmentation detected by staining with Hoechst 33432. The treatment for each sample is indicated above each panel.

this study were free of E1+ adenovirus and endotoxin. Particle:plaque ratios were between 30:1 and 100:1.

To test the induction of the *bax* gene in cultured mammalian cells by adenovirus-mediated gene cotransfer, human lung carcinoma cell lines H1299 and A549 were infected with Ad/GT-Bax and Ad/PGK-GV16[10] at a vector ratio of 2:1 and at a total multiplicity of infection (MOI) of 900 and 1500 particles, respectively. Cells were treated with PBS or infected either with Ad/GT-Bax plus Ad/CMV-GFP (a gift from Dr TJ Liu at our institution),[12] or with Ad/GT-LacZ[10] plus Ad/PGK-GV16 at the same vector ratio, and MOIs were used as controls. Cells were harvested 24 h after the treatment and their lysates subjected to Western blot analysis. Although background levels of the bax protein expression differed between H1299 and A549 cells and although the treatment with control vectors did not increase those background levels, a strong induction of bax expression was detected in both cell lines when they were treated with Ad/GT-Bax plus Ad/PGK-GV16 (Figure 1). The induction was observed to be 67.2- and 8.7-fold in H1299 and A549 cells, respectively, when the densities of the bax-specific bands were quantified and normalized to the density of β-actin bands.

Overexpression of the *bax* gene has been demonstrated to induce the release of Cyt c from mitochondria, which leads first to cleavage of caspase-3/CPP32 followed by cleavage of poly (ADP ribose)polymerase (PARP).[13] To test whether the induction of *bax* expression by adenovirus-mediated gene codelivery would similarly trigger apoptosis in H1299 and A549 cells, samples of the same cell lysate from the above-mentioned experiments were subjected to Western blot analysis of the cleavage of caspase-3 and PARP. The cleavage of caspase-3 into a 17-kDa fragment and PARP into a 85-kDa fragment was detected in cells treated with Ad/GT-Bax plus Ad/PGK-GV16 but not in cells from any other experimental groups (Figure 2a). To document the apoptosis in these cells further, H1299 and A549 cells were treated with various vectors at a total MOI of 900 and 1500 particles, respectively, as mentioned above, and observed for cytopathology and morphology changes at 48 h after treatment. Over 80% of the cells treated with Ad/GT-Bax plus Ad/PGK-GV16 showed signs of cytopathology, and became rounded and detached, whereas the cells in all other treated groups remained in monolayers with normal morphology. Nuclear fragmentation, a hallmark of cell apoptosis, was detected only in cells treated with Ad/GT-Bax plus Ad/PGK-GV16 (Figure 2b), suggesting that *bax* expression by this system activated not only the caspase cascade, but ultimately extensive apoptosis in these human lung cancer cell lines.

To test whether *bax* gene expression could be similarly induced by adenovirus-mediated gene codelivery *in vivo*, adult Balb/c mice were infused via their tail veins with PBS, Ad/GT-Bax plus Ad/CMV-GFP, Ad/GT-Bax plus Ad/PGK-GV16, or Ad/GT-LacZ plus Ad/PGK-GV16 at a total vector dose of 6×10^{10} particles per mouse and a vector ratio of 2:1. Mice were then killed at 24 h after treatment, and liver samples were harvested for Western blot analysis and histopathological examination. Western blot analysis showed a 14-fold increase in bax protein levels in animals treated with Ad/GT-Bax plus Ad/PGK-GV16, but only background level in all other treatment groups (Figure 3a). These results clearly demonstrated that the Ad/GT-Bax plus Ad/PGK-GV16 strictly regulated *bax* expression by expressing GAL4/VP16 protein even *in vivo*. Expression of the *bax* gene also induced typical apoptosis in normal liver cells, as revealed by nuclear fragmentation and condensation in hematoxylin- and eosin-stained liver sections (Figure 3b). Of note, administration of high-dose E1-deleted adenoviral vector itself will cause histopathological changes in hepatocytes, including ballooning, degeneration and apoptosis (1–3% of hepatocytes) that usually occur 2–3 days after the treatment and are followed by lymphocytic infiltration.[14,15] However, examination of the above liver sections showed that apoptosis accounted for over 60% of hepatocytes in the animals treated with Ad/GT-Bax plus Ad/PGK-

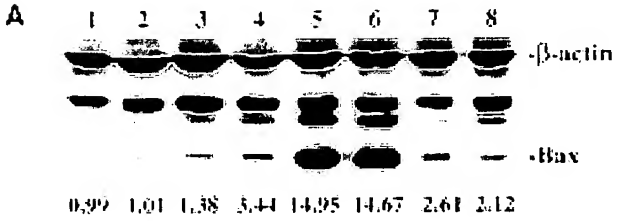


Figure 3 In vivo induction of bax gene expression. The data represent similar results from two separate in vivo experiments with five mice per group. (A) Western blot analysis of bax protein expression in livers from Balb/c mice treated with PBS (lanes 1 and 2), Ad/GT-Bax + Ad/CMV-GFP (lanes 3 and 4), Ad/GT-Bax + Ad/PGK-GV16 (lanes 5 and 6), and Ad/GT-LacZ + Ad/CMV-GV16 (lanes 7 and 8). The bax: β-actin ratio for each sample is listed under each lane. Each ratio is expressed in terms relative to the ratio in PBS-treated mice, which was arbitrarily set at 1. (B) Nuclear fragmentation detected by hematoxylin and eosin staining of liver sections from mice treated with (a) PBS, (b) Ad/GT-Bax + Ad/CMV-GFP, (c) Ad/GT-Bax + Ad/PGK-GV16, and (d) Ad/GT-LacZ + Ad/CMV-GV16.

Figure 3 Continued.

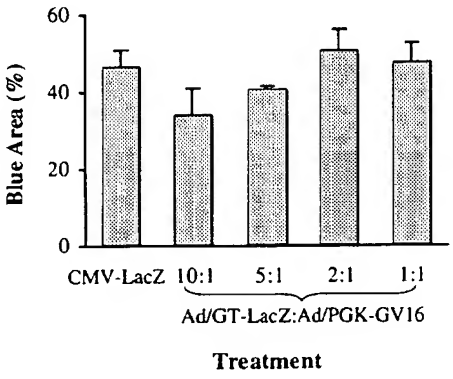


Figure 4 Induction of LacZ by adenovirus-mediated gene codelivery. H1299 cells were transduced by Ad/CMV-LacZ or Ad/GT-LacZ + Ad/PGK-GV16 at a total MOI of 10 p.f.u. and varying ratios of Ad/GT-LacZ:Ad/PKG-GV16 as indicated at the bottom of the graph. Cells were stained with X gal 24 h after infection. The percentage of blue area was determined with the aid of Optimas software. The values represent mean + s.d. of four fields from two duplicated assays. No blue cell was detected in cells treated with PBS, Ad/PGK-GV16 or Ad/GT-LacZ alone at the MOI of 10 p.f.u.

GV16 but only less than 1% of hepatocytes in all the other controls. The massive apoptosis of hepatocytes within 24 h after vector administration that was observed in all animals of the *bax*-expressing group but in none of the other controls indicated that it was mediated by *bax* overexpression. Together, these results demonstrated that adenovirus-mediated gene cotransfer could produce sufficient bax expression and induce apoptosis *in vivo*.

In a separate study, we have addressed the question of transduction efficiency and the optimal ratio of the two vectors in the binary adenoviral vector system. It is possible that the transduction efficiency may be reduced in the binary system because only the cells dually transduced with both vectors will express the desired transgene. To determine transduction efficiency and the optimal ratio of two vectors, H1299 cells were infected with Ad/GT-LacZ, Ad/CMV-LacZ, Ad/PGK-GV16 and Ad/GT-LacZ plus Ad/PGK-GV16 at a ratio of 10:1, 5:1, 2:1 and 1:1. A total MOI of 10 (p.f.u.) was used in each treatment. The cells were fixed 24 h after the infection and the transduction efficiency was determined by X-gal staining (Figure 4). No blue cells were detected in cells infected with either Ad/PGK-GV16 or Ad/GT-LacZ. Transduction efficiency as determined by the percentage of blue areas with the Optimas software were not significantly different among cells treated with Ad/CMV-LacZ, Ad/GT-LacZ plus Ad/PGK-GV16 at the ratio of 2:1 and 1:1 ($P \geqslant 0.24$). Transduction efficiencies were, however, lower in groups treated with Ad/GT-LacZ plus Ad/PGK-GV16 at the ratio of 10:1 and 5:1 ($P \leqslant 0.033$). These results suggest that transduction efficiency is not hampered in the binary vector system at the optimal ratio of two vectors. It is likely that, even in the case of Ad/CMV-LacZ, more than one copy of vector genome per cell will be required to produce a visible blue color by X-gal staining.

In conclusion, our successful induction of *bax* gene expression and of apoptosis in target cells by using a GAL4 gene regulatory system and adenovirus-mediated gene cotransfer suggests that it may provide an alternative approach to constructing adenoviral vectors expressing proapoptotic and/or cytotoxic genes. The fact that the transduction efficiency was not hampered by using two vectors suggested that the vector dose may not be increased in the binary vector system in order to achieve a similar therapeutic effect as that of a single vector system. Our results also suggest that overexpression of the *bax* gene may cause toxic side-effects, as massive hepatocyte apoptosis was observed after intravenous infusion of the *bax* expressing vectors. However, because the expression of the *bax* gene is fully dependent on the presence of the GAL4/VP16 fusion protein, it may be that the expression of a proapoptotic gene targeted to certain target cells can be achieved by using a tissue- or cell-type specific promoter to drive the GAL4/VP16 fusion protein. Nevertheless, since PGK is a ubiquitous promoter, induction of *bax* gene expression by Ad/PGK-GV16 should be useful for testing therapeutic values of the gene in a variety of cancer cells.

Acknowledgements

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References

1 Kovesdi I *et al*. Adenoviral vectors for gene transfer. *Curr Opin Biotech* 1997; **8**: 583–589.
2 Yeh P, Perricaudet M. Advances in adenoviral vectors: from genetic engineering to their biology. *FASEB J* 1997; **11**: 615–623.
3 Larregina AT *et al*. FasL induces Fas/Apol-mediated apoptosis in human embryonic kidney 293 cells routinely used to generate E1-deleted adenoviral vectors. *Gene Therapy* 1998; **5**: 563–568.
4 Arai H, Gordon D, Nabel EG, Nabel GJ. Gene transfer of Fas ligand induces tumor regression *in vivo*. *Proc Natl Acad Sci USA* 1997; **94**: 13862–13867.
5 Okuyama T *et al*. Efficient Fas-ligand gene expression in rodent liver after intravenous injection of a recombinant adenovirus by the use of a Cre-mediated switching system. *Gene Therapy* 1998; **5**: 1047–1053.
6 Shinoura N *et al*. Construction, propagation, and titer estimation of recombinant adenoviruses carrying proapoptotic genes. *Hum Gene Ther* 1998; **9**: 2683–2689.
7 Apte SS, Mattei MG, Olsen BR. Mapping of the human BAX gene to chromosome 19q13.3-q13.4 and isolation of a novel alternatively spliced transcript, BAX delta. *Genomics* 1995; **26**: 592–594.
8 Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* 1993; **74**: 609–619.
9 Coll JL *et al*. Antitumor activity of bax and p53 naked gene transfer in lung cancer: *in vitro* and *in vivo* analysis. *Hum Gene Ther* 1998; **9**: 2063–2074.
10 Fang B, Ji L, Bouvet M, Roth JA. Evaluation of GAL4/TATA *in vivo*. Induction of transgene expression by adenovirally mediated gene codelivery. *J Biol Chem* 1998; **273**: 4972–4975.
11 Zhang WW *et al*. Generation and identification of recombinant adenovirus by liposome-mediated transfection and PCR analysis. *Biotechniques* 1993; **15**: 868–872.
12 Fueyo J *et al*. Overexpression of E2F-1 in glioma triggers apoptosis and suppresses tumor growth *in vitro* and *in vivo*. *Nature Med* 1998; **4**: 685–690.

13 Tewari M *et al*. Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly (ADP-ribose) polymerase. *Cell* 1995; **81**: 801–809.
14 Lieber A *et al*. The role of Kupffer cell activation and viral gene expression in early liver toxicity after infusion of recombinant adenovirus vectors. *J Virol* 1997; **71**: 8798–8807.
15 Yang Y *et al*. Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy. *Proc Natl Acad Sci USA* 1994; **91**: 4407–4411.